Assemblies and Aggregates I

300-Pos Board B55
A View to a Kill: T6SS-Mediated Cell Killing Visualized by Fluorescence Microscopy
Jacqueline Corbitt[1], Michele Leroux[2], Joseph Mougous[3], Paul Wiggins[4].
[1]Physics, University of Washington, Seattle, WA, USA, [2]Microbiology, University of Washington, Seattle, WA, USA, [3]Molecular and Cellular Biology, University of Washington, Seattle, WA, USA, [4]Physics & Bioengineering, University of Washington, Seattle, WA, USA.
The Type Six Secretion System (T6SS) is a bacterial toxin-delivery system targeting bacterial cells which neighbor the donor, promoting recipient cell death. The T6SS is widely conserved among Gram-negative bacteria and may be a central determinant in bacterial fitness in polymicrobial communities of particular relevance to chronic infection. Sequence homology of secretion system components to the T4 bacteriophage tail spike, cryoEM reconstructions of the secretion system and fluorescence imaging are all consistent with a dynamic mechanism of secretion. The complex system, which is composed of at least 15 proteins, forms a puncturing apparatus/delivery system which uses a donor protein filament to puncture the recipient cell wall to deliver protein toxins. Using quantitative imaging analysis of multiple fluorescent fusions, we present a detailed characterization of T6SS system dynamics visualized in single cells in multiple bacterial species, developing a model of T6SS function. We present quantitative measurements of the dynamics of the secretion system - from the assembly to contraction to disassembly - in conjunction with quantitative measures of system function, including recipient cell lysis.

301-Pos Board B56
A FRET-Based Method for Measurement of Yeast Septin Filament Formation In Vitro
Elizabeth Booth[1], Eleanor Vane[2], Jeremy Thorner[1].
[1]Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA, USA, [2]Clarkson University, Potsdam, NY, USA.
Septins are a family of GTP-binding cytoskeletal proteins conserved in all eukaryotes (except higher plants) that have roles in erecting diffusion barriers, affecting membrane curvature, and providing protein scaffolding. Budding yeast (Saccaromyces cerevisiae) is a convenient organism for investigating septin organization and function because of the wide variety of tools available. Because septins are conserved, understanding the principles that dictate how the yeast proteins organize should provide insight into septin structure and function in higher eukaryotes, including humans. Single-particle EM analysis has revealed that septin subunits associate to form linear apolar hetero-octameric rods. In vitro, septin rods polymerize end-on-end into long, straight paired filament. However, no facile method has existed for studying the polymerization of septin hetero-octamers in vitro in real time. We have previously generated yeast septin complexes wherein all endogenous Cys residues were eliminated by site-directed mutagenesis, except either one native Cys (C43) or an introduced E294C mutation in Cdc11. Because Cdc11 is the terminal subunit in hetero-octamers, derivatization of the single Cys residue with organic dyes should permit the use of Förster resonance energy transfer (FRET) to monitor filament assembly. In initial experiments conducted with mixtures of donor- and acceptor-labeled hetero-octamers, the expected FRET is exhibited under conditions that favor polymerization (low salt), but not under conditions known to prevent polymerization (high salt). Furthermore, the amount of FRET observed depends on the input concentrations and relative ratios of the donor and acceptor dye-labeled rods. These findings are fully consistent with previous observations made by EM and indicate that the observed FRET is providing a reliable solution-based assay for the end-to-end assembly of hetero-octamers into filaments. This assay can now be exploited to study the effects of septin concentration, cofactors (e.g., guanine nucleotides) and septin-interacting proteins.

302-Pos Board B57
Investigating the Mechanism of Collagen Self-Assembly with Microrheology
Marjan Shayegan, Tuba Altindal, **Nancy R. Forde**.
Simon Fraser University, Burnaby, BC, Canada.
Collagen is the predominant protein in vertebrates, where it comprises more than 1/4 of the total protein and performs structural and mechanical tasks in the extracellular matrix and connective tissues. Formed intracellularly as a triple helical protein, collagen undergoes self-assembly extracellularly into fibrils, which in turn contribute to higher-order structures. Self-assembly of collagen into well-ordered fibrils can be replicated with appropriate solvent conditions *in vitro*, implying that the keys to self-association are found within its sequence.
We perform microrheology experiments on collagen systems, using optically trapped particles as probes of local, microscale viscoelasticity. Our initial experiments investigated the time-dependent development of viscoelastic heterogeneity in collagen systems as they undergo self-assembly from proteins in solution into fibrillar structures.[1] Here, we examine alterations in collagen's chemical composition that influence its self-assembly. We find that the removal of collagen's nonhelical "telopeptide" ends significantly reduces elasticity of collagen solutions at timescales from ~10 msec to ~1 sec. While in the acidic solutions of these experiments collagen does not assemble into fibrils, our results nonetheless provide insight into the catalytic mechanism of these short domains on fibril formation. The removal of telopeptides has long been known to slow down fibril formation, and telopeptides have previously been postulated to transiently associate with other chains in solution,[2] thus providing "docking points" from which lateral assembly of collagen triple helices into fibrils can proceed. Here, our microrheology experiments provide direct evidence of increased strength and duration of interprotein contact arising from the presence of telopeptides, critical in catalyzing self-assembly of fibrillar collagen systems.
[1] M. Shayegan and N.R. Forde, *PLoS ONE***8**, e70590 (2013)
[2] N. Kuznetsova and S. Leikin, *J. Biol. Chem.***274**, 36083 (1999); D.J. Prockop and A. Fertala, *J. Biol. Chem.***273**, 15598 (1998).

303-Pos Board B58
Atomic Force Microscopy Imaging Reveals Structural and Mechanical Properties of Dissociated Hemocyanins by Temperature
Camilo Navarrete[1], Javiera Villar[2], Yessenia Aguilar[2], Ricardo Cabrera[2], Nelson P. Barrera[1].
[1]Universidad Católica de Chile, Santiago, Chile, [2]Universidad de Chile, Santiago, Chile.
Hemocyanins are protein complexes responsible for the oxygen transport in arthropods and molluscs. These proteins contain a copper binuclear active site and are assembled as four hexamers with an overall size around 1,7MDa. Using Atomic force microscopy (AFM) imaging on purified hemocyanins from Grammostola rosea and Paraphysa sp, we have observed that both species showed a similar tetrameric assembly and molecular volume. Given that Paraphysa sp survives at lower temperatures than Grammostola rosea we also study by AFM the dissociated hemocyanin complexes obtained by freeze-defreeze cycles. The gel filtration pattern of these complexes indicates a similar behaviour between both species. In addition, by force spectroscopy measurements on each hexameric component, we have determined their elasticity behaviour. Altogether these demonstrate that AFM is a powerful tool to record simultaneously both kinetics and mechanical properties of hemocyanin. Funded by Fondecyt Grant 1120169, Millennium Nucleus Grant P10-035F and Anillo Grant ACT1108.

304-Pos Board B59
Tripeptides Screening Report: Proline is Important for Aβ Fibrils Depolymerization
Katarina Siposova[1], Man Hoang Viet[2], Mai Suan Li[2], Zuzana Bednarikova[1,3], Andrea Antosova[1], Truc Trang Nguyen[4], Zuzana Gazova[1,5].
[1]Department of Biophysics, Institute of Experimental Physics SAS, Kosice, Slovakia, [2]Institute of Physics, Polish Academy of Sciences, Warsaw, Poland, [3]Department of Biochemistry, Faculty of Science, P. J. Safarik University, Kosice, Slovakia, [4]Institute for Computational Science and Technology, Ho Chi Minh City, Viet Nam, [5]Department of Medical and Clinical Biochemistry and LABMED, Faculty of Medicine, P.J. Safarik University, Kosice, Slovakia.
Alzheimer's disease (AD) is the most frequent form of dementia among the elderly and is associated with the extracellular A-beta amyloid deposits in brain. One of the most straightforward approaches for finding a treatment of Alzheimer's disease is targeting of Aβ amyloid fibrillization. In our work we performed comprehensive study of the effect of all possible three-amino acid peptides (8000 tripeptides in total) on Aβ fibrils depolymerization using the molecular modeling and we analyzed the binding affinity of tripeptides to Aβ fibrils. By both docking and MM-PBSA methods tripeptides containing Proline and aromatic amino acids were identified as potentially the most effective. The ability of selected tripeptides to destroy amyloid fibrils was also investigated experimentally by ThT fluorescence assay and AFM microscopy in vitro. Using in silico and in vitro methods we have showed that selected tripeptides can destroy preformed Aβ fibrils.

The molecular binding approach suggests that tripeptides are preferably located near hydrophobic residues of Aβ fibrils. The DC50 values determined from dose-response curves were found in micromolar range, the lowest values were found for tripeptides containing Proline. Experimental results confirmed theoretically predicted significance of Proline in tripeptide sequence.

Acknowledgement: This work was supported by grants VEGA 0181, No 2011/01/B/NZ1/01622, ESF 26110230061 and 26110230097, APVV 0171-10 and VVGS 98/13-14.

305-Pos Board B60

Efflux Time Courses of Cytosolic Proteins from Rabbit Skinned Muscle Fibers Reflect Dissociation of Enzyme Complexes

David Maughan[1], Brian Carlson[2], Jim Vigoreaux[3].
[1]Molecular Physiology/Biophysics, University of Vermont, Burlington, VT, USA, [2]Biotechnology and Bioengineering Center, Milwaukee WI USA, Medical College of Wisconsin, Milwaukee, WI, USA, [3]Biology, University of Vermont, Burlington, VT, USA.

In many multi-system metabolic diseases characterized by mitochondrial dysfunction (e.g. Myalgic Encephalomyelitis), glycolysis appears to be an important compensatory pathway for generating ATP. It is postulated that glycolytic enzymes form a complex that enhances the rate of ATP production through substrate channeling of metabolic product intermediates. To search for evidence of a reduced diffusion coefficient indicative of supramolecular complexes, we examined the efflux of endogenous glycolytic enzymes from rabbit psoas muscle fibers. Single fiber segments were skinned in oil and transferred to physiological salt solution. Cytosolic proteins that diffused into the solution were separated by gel electrophoresis and compared to load-matched standards for quantitative analysis. A radial diffusion model incorporating the dissociation and dissipation of supramolecular complexes accounts for an initial lag and subsequent efflux of glycolytic enzymes. The model includes terms representing protein crowding, myofilament lattice hindrance, and cytomatrix binding. Optimization of model to data returned estimates of apparent diffusion coefficients that were very low at the onset of diffusion ($\sim 10^{-10}$ cm^2 s^{-1}) but increased with time as cytosolic protein density decreased. The initial values are consistent with the presence of complexes *in situ*; higher later values (e.g., 0.2×10^{-7} cm^2 s^{-1} for phosphofructose kinase), with molecular sieving and transient binding of dissociated proteins. Channeling of metabolic intermediates via enzyme complexes may enhance production of ATP at rates beyond that possible with randomly distributed enzymes, thereby matching supply with demand. Metabolic channeling may allow glycolysis to better compensate for reduced ATP production in aerobic metabolic diseases.

306-Pos Board B61

Phase Diagram to Illustrate Protein Aggregation Profile and Conditions

Bin Li[1], Siqi Li[2], Yang Cao[2], Shaohua Xu[2].
[1]Florida Institute of Technology, Melbourne, FL, USA, [2]Department of Biological Sciences, Florida Institute of Technology, Melbourne, FL, USA.

Protein self-assembly and formation of amyloid fibers and/or amorphous aggregates is an early event in numerous human diseases, such as Alzheimer's disease, Parkinson's disease, and cataracts. Identification of the structural features generated in the aggregation process, especially under conditions similar to the tissue's viscous and crowded environment, helps to elucidate the mechanism of protein aggregation and the pathogenesis of these diseases. By systematically testing a broad range of conditions and construction of a 3-dimensional phase diagram, we identified the pH, salt, lysozyme concentration, and incubation time, for lysozyme to form amyloid fibers, amorphous aggregates, and gels. We examined the effect of viscosity and molecular crowding on lysozymes' aggregation profile. We characterized the aggregates by use of AFM, TEM, FPLC, and Thioflavin T binding assays, and found that amyloid fibers are formed between pH 2.0 and 3.0, amorphous aggregates at pH 3.5 and above. Glycerol or polyethylene glycol inhibits fiber formation. Gels are formed when fiber concentration is high, and the presence of glycerol or polyethylene glycol lowers the minimum fiber concentration required for gelation. Salt or shaking promotes amyloid fiber formation and shortens the time needed for gelation. Colloidal spheres are present in amyloid fiber solutions, as predicted by the linear colloidal aggregation model we introduced previously. The phase diagrams provide a comprehensive and clear picture of the relation between various aggregates and the conditions for their formation.

307-Pos Board B62

In Vitro Interactions Between Amyloid Beta and Islet Amyloid Polypeptide

Leah Vandiver.
Biomedical Engineering, Washington University in St. Louis, St. Louis, MO, USA.

Alzheimer's disease and type II diabetes mellitus are two prevalent protein misfolding diseases. Their co-morbidity has raised questions regarding potential interactions between the peptides that are implicated in each disease state. Islet amyloid polypeptide (IAPP) aggregates in type II diabetes mellitus while amyloid beta 1-42 (Aβ42) aggregates in Alzheimer's disease. Interactions between these amyloidogenic peptides may result in coaggregation that could exacerbate the cellular toxicity associated with each disease by increasing the amount of toxic oligomers over less toxic mature fibrils.

We tested this hypothesis *in vitro* by studying aggregation kinetics, aggregate morphology, stability and cytotoxicity of IAPP / Aβ42 mixtures. Tetramethylrhodamine (TMR)-labeled Aβ42 was incubated with excess AlexaFluor 488 (A488)-labeled IAPP which revealed through colocalization analysis that both peptides coaggregate. A complimentary experiment with excess TMR-labeled Aβ42 showed similar results. We then monitored aggregation kinetics of IAPP and Aβ42 through Thioflavin T fluorescence. Equimolar mixtures of Aβ42 and IAPP displayed aggregation kinetics intermediate of Aβ42 and IAPP. While IAPP prolonged the lag-phase of Aβ42, the opposite was not observed. At the same time, the presence of equimolar or excess IAPP diminished the SDS-resistance of fibrillar Aβ42 aggregates. We then added aggregation intermediates corresponding to the lag phase, early and late growth phases, and the plateau phase to human neuroblastoma (SH-EP) cells to test for cytotoxicity. Surprisingly, the cytotoxicity of Aβ42 / IAPP mixtures was intermediate to that of Aβ and IAPP alone. Taken together, these results indicate a molecular interaction exists between these two disease-relevant peptides and may provide a link between type II diabetes mellitus and Alzheimer's disease but does not support a model in which co-aggregation with IAPP directly exacerbates Aβ toxicity by increasing oligomer formation.

308-Pos Board B63

NADH is an Endogenous Reporter for Alpha-Synuclein Aggregation in Live Cells

Nicoletta Plotegher[1], Chiara Stringari[2], Sohail Jahid[3], Enrico Gratton[3], Luigi Bubacco[1].
[1]Department of Biology, University of Padova, Padova, Italy, [2]Laboratory for Optics and Biosciences, École Polytechnique, Paris, France, [3]Laboratory for Fluorescence Dynamics, University of California, Irvine, CA, USA.

Alpha-synuclein aggregation is amply investigated for its involvement in Parkinson's disease etiopathogenesis. It has been shown that alpha-synuclein monomers, under pathological conditions, self-assembly to form oligomeric species that further aggregate into amyloid fibrils. Alpha-synuclein fibrils are the main constituent of Lewy Bodies, which are one of the characteristic hallmarks of Parkinson's disease.

Alpha-synuclein aggregation is studied in vitro and in cellular models with the aim to correlate toxicity mechanisms to defined aggregation products. However, the characterization of the aggregation process in cells is a difficult task that typically needs cell lysis or fixation, or the use of exogenous dyes.

Moreover, several different toxic mechanisms were ascribed to alpha-synuclein aggregates, i.e. clearance mechanisms impairment, mitochondrial dysfunctions, oxidative stress, neuroinflammation. In particular, mitochondria seem to be a target for alpha-synuclein to exert its toxicity. Several independent results suggested that alpha-synuclein overexpression and/or aggregation may cause impairment of cellular metabolism due to mitochondrial fragmentation and complex I dysfunction.

On these premises, we report here the results obtained from the characterization of NADH fluorescence properties variation in vitro and in cell models during alpha-synuclein aggregation.

The application of the phasor approach for the study of NADH fluorescence lifetime and spectra allowed the determination of specific variation in the NADH fluorescence properties correlated to alpha-synuclein oligomerization and amyloid fibrils formation in vitro and in live cells.

The results presented here suggest that alpha-synuclein aggregation may be associated to impairment in cell metabolism due to damage to complex I in mitochondria and disruption of NADH and NAD^+ equilibrium. Moreover, NADH can be used as an endogenous fluorescence reporter for alpha-synuclein aggregation in vitro and in cellular models.

(Work supported in part by NIH grants P50 GM076516 and P41 GM103540, and by Aldo Gini Foundation.)